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Research Article

RESEARCH ARTICLE

Active bacterioplankton community response to dissolved 'free' deoxyribonucleic acid (dDNA) in surface coastal marine waters

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One sentence summary: This study identifies specific active bacterioplankton in surface coastal marine waters that utilise dissolved 'cell free' DNA by assessing responses to DNA addition and DNA-uptake.

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ABSTRACT

Seawater contains dissolved 'free' DNA (dDNA) that is part of a larger <0.2 µm pool of DNA (D-DNA) including viruses and uncharacterised bound DNA. Previous studies have shown that bacterioplankton readily degrade dDNA, and culture-based approaches have identified several potential dDNA-utilising taxa. This study characterised the seasonal variation in D-DNA concentrations at Station L4, a coastal marine observatory in the Western English Channel, and linked changes in concentration to cognate physicochemical and biological factors. The impact of dDNA addition on active bacterioplankton communities at Station L4 was then determined using 16S rRNA high-throughput sequencing and RNA Stable Isotope Probing (RNA SIP) with ¹³C-labelled diatom-derived dDNA. Compared to other major bacterioplankton orders, the Rhodobacterales actively responded to dDNA additions in amended microcosms and RNA SIP identified two Rhodobacterales populations most closely associated with the genera *Halocynthiaibacter* and *Sulfitobacter* that assimilated the ¹³C-labelled dDNA. Here we demonstrate that dDNA is a source of dissolved organic carbon for some members of the major bacterioplankton group the Marine Roseobacter Clade. This study enhances our understanding of roles of specific bacterioplankton taxa in dissolved organic matter cycling in coastal waters with potential implications for nitrogen and phosphorus regeneration processes.

Keywords: DNA; bacterioplankton; stable-isotope probing; DON; DOC; DOP

INTRODUCTION

Phytoplankton growth, death and lysis releases large amounts of dissolved organic matter (DOM) (Agustí and Duarte 2013),

which is a vital resource for heterotrophic bacterioplankton through the microbial loop (Azam et al. 1983). In addition to carbon for bacterioplankton growth, DOM also provides accessible nitrogen and phosphorus, particularly after

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phytoplankton blooms when inorganic nutrients are at lower concentrations (Smyth et al. 2010). The dynamics of the production and subsequent bacterioplankton cycling of DOM remain poorly understood, in part, because many of the complex specific DOM compounds are currently poorly characterised (McCarthy, Hedges and Benner 1993; McCarthy et al. 1997; Benner 2002).

A distinct component of the seawater DOM pool are nucleic acids such as deoxyribonucleic acid (DNA). Of the total seawater DNA pool that is operationally defined as $<0.2 \mu\text{m}$ (D-DNA), much is dissolved 'free' DNA (dDNA) as well as DNA viruses and uncharacterised bound DNA (Brum 2005). DNA is ubiquitous throughout the marine environment with variable concentrations (Table 1) (DeFlaun, Paul and Jeffrey 1987; Karl and Bailiff 1989). Studies in the Aegean Sea have shown that seawater DNA concentrations are higher during summer months (June to October) than winter months (November to May), coinciding with periods of increased primary productivity (Weinbauer, Fuks and Peduzzi 1993; Weinbauer et al. 1995). Seawater DNA concentrations are also higher in surface coastal waters than in open ocean and deeper waters (DeFlaun, Paul and Jeffrey 1987; Boehme et al. 1993; Weinbauer et al. 1995; Brum 2005), suggesting that DNA has a strong temporal variation related to plankton biomass and activity.

Previous studies have shown that dDNA is readily degraded and assimilated by marine bacterioplankton (Maeda and Taga 1974; Paul, Jeffrey and DeFlaun 1987; Paul, DeFlaun and Jeffrey 1988); however, few studies have identified specific bacterioplankton taxa actively involved in the cycling of dDNA. Lennon (2007) isolated dDNA-utilising bacteria from seawater collected from Eel Pond (Woods Hole, MA) using dDNA-containing growth media and identified several genera, including *Vibrio*, *Alteromonas*, *Pseudoalteromonas* and *Roseobacter*. Comparison between the isolates indicated that different taxa are adapted to better utilise either low-molecular-weight or high-molecular weight dDNA pools depending on the original isolation conditions.

From a biogeochemical perspective, nucleic acids and associated degradation products (e.g. purines and pyrimidines) make important contributions to seawater carbon, nitrogen and phosphorus DOM pools, with C:N:P ratios that exceed that of typical 'Redfield' plankton (Berman and Bronk 2003; Karl and Björkman 2015). Bacterioplankton cycling of these compounds can therefore result in nitrogen and phosphorus regeneration processes taking place that produce substrates that are more widely accessible to the plankton communities, such as urea (Berg and Jorgensen 2006). For example, studies at Station ALOHA in the North Pacific Subtropical Gyre have suggested that dDNA can provide a major component of the total biologically available phosphorus demand throughout the water column (Brum 2005).

The aims of this study were to quantify seawater D-DNA concentrations in surface coastal waters at the Station L4 sampling site in the Western English Channel over a spring-summer transition and to assess the relationships between changes in concentration with physiochemical and biological factors. This study focused on the dDNA component of the D-DNA pool by identifying specific bacterioplankton taxa able to utilise phytoplankton-derived dDNA using dDNA-amended seawater microcosms and RNA Stable Isotope Probing (RNA SIP) with ^{13}C -labelled diatom dDNA.

MATERIALS AND METHODS

Seawater D-DNA quantification

Seawater samples were collected weekly between 04/03/2014 and 21/06/2014 from Station L4 (Lat 50.15 Lon -4.13) in the Western English Channel (Fig. S1, Supporting Information) from 5 m depth. 50 mL samples ($n = 3$) were gently filtered through a $0.2 \mu\text{m}$ Minisart® cellulose acetate membrane filter (Sartorius, UK) into sterile aged (> 5 year old) plastic bottles containing $50 \mu\text{L}$ 1 M tetrasodium ethylenediaminetetraacetic acid (EDTA) (Brum, Steward and Karl 2004), stored in the dark at 4°C and returned to the laboratory for analysis within 2 h.

D-DNA (combined dissolved 'free' DNA, DNA viruses and uncharacterised bound DNA) was quantified fluorometrically using the method described by Brum, Steward and Karl (2004). 15 mL samples were concentrated using Amicon Ultra Centrifugal Filter units (ultra-15 MWCO 10k Da, Sigma Aldrich, UK) centrifuged for between 10 and 20 min at $4000 g$ and 25°C , reducing the volume to 1 mL. 10 mL Tris-EDTA buffer (TE buffer: 10 mM Tris-HCl, 1 mM disodium EDTA, pH 7.5, autoclaved) was added to the concentrate before further centrifugation to a final volume of $250 \mu\text{L}$. The concentrated D-DNA was quantified using the QuantiFluor dsDNA System (Promega, UK) using a slight modification of the manufacturer's instructions, with $5 \mu\text{L}$ concentrated DNA samples added to $45 \mu\text{L}$ of DNA-binding dye and quantified using a calibrated QuantiFluor® (Promega, UK).

Phytoplankton and bacterioplankton abundance

Seawater samples were also collected from Station L4 from a depth of 10 m using a 10 L Niskin bottle, with 200 mL removed and immediately preserved with 2% (final concentration) acid-Lugol's iodine solution (Thronsdon 1978) and 200 mL preserved with neutral formaldehyde (4% final concentration) (Widdicombe et al. 2010). Samples were analysed using the Utermöhl technique (Utermöhl 1931) according to guidance procedures within 'Water quality—Guidance standard for routine microscopic surveys of phytoplankton using inverted microscopy (Utermöhl technique)' (BS EN 15 204:2006). Samples were acclimatised to room temperature to ensure a random distribution of cells in the settlement chambers. Following cell re-suspension and separation through gentle rotation of sample bottles in a figure-of-eight movement, a subsample volume of either 50 or 100 mL (depending on cell density) was transferred to a plankton settling chamber. Cells were identified, where possible, to species level according to the published literature. Abundance of high and low nucleic acid bacteria were assessed using flow cytometry from the un-preserved seawater samples using protocols outlined in Tarran and Bruun (2015).

^{13}C -labelled dDNA production

Chaetoceros contortus PLY550 was selected as a candidate for dDNA production because the genus *Chaetoceros* is bloom forming at Station L4 (Widdicombe et al. 2010). Axenic cultures (1 L) of *C. contortus* were grown in artificial seawater media (Berges, Franklin and Harrison 2001) with f/2 nutrients (Guillard and Ryther 1962). To produce ^{13}C -labelled dDNA, the media was modified by adding $\text{NaH}^{13}\text{CO}_3$ (5 mM). The cultures were maintained sealed and incubated at 15°C for 14 days under a 16:8 hours light dark cycle regime at an intensity of $85.3 \mu\text{mol photons s}^{-1} \text{m}^{-2}$, with daily mixing by inversion.

Table 1. Dissolved DNA (D-DNA) concentrations in seawater showing the range of values obtained in this study and values from other studies.

Location	D-DNA range ($\mu\text{g L}^{-1}$)	Reference
L4, Plymouth, (U.K)	0.09–1.50	This study
N. Adriatic Sea (Europe)	2.9–25.8	Weinbauer, Fuks and Peduzzi (1993)
N. Adriatic Sea (Europe)	3.1–26.5	Weinbauer et al. (1995)
N. Adriatic Sea (Europe)	0.05–0.8	Breter et al. (1977)
Tampa Bay & Charlotte Harbour (Florida)	10–19	DeFlaun, Paul and Jeffrey (1987)
Tampa Bay (Florida)	11.9–16.8	Boehme et al. (1993)
Gulf of Mexico—Oceanic	3.5–6.7	Boehme et al. (1993)
Gulf of Mexico—Coastal	5–15	DeFlaun, Paul and Davis (1986)
Kaneohe Bay (Hawaii)	3.41	Brum et al. (2004)
Kaneohe Bay (Hawaii)	2.66–3.15	Karl and Bailiff (1989)
Ala Moana Beach (Hawaii)	19–21	Karl and Bailiff (1989)
Magic Island (Hawaii)	14–19	Karl and Bailiff (1989)
Mamala Bay (Hawaii)	4.7	Karl and Bailiff (1989)
Tokyo Bay (Japan)	9–19	Maruyama, Oda and Higashihara (1993)
Bombay Harbour (India)	13.4–80.6	Pillai and Ganguly (1970)

Cultures were harvested by centrifugation at 4000 *g* for 20 min and the cell pellets stored at -80°C . dDNA was extracted from the cell pellets using the DNeasy Blood and Tissue Kit (Qiagen, UK). Resulting dDNA extracts were RNase treated to remove potential co-extracted RNA (RNase 1, Promega, UK) as per the manufacturer's instructions. The dDNA solution was made up to 1 mL using Nuclease free water and concentrated to 250 μL in Amicon Ultra Centrifugal Filter units (ultra-15 MWCO 10k Da) by centrifuging at 4000 *g* for 5–10 min. The resulting 10k Da fraction (consistent with the high molecular weight fraction quantified from environmental samples) was recovered and stored at -80°C .

Experimental setup

Surface seawater (5 m depth, 60 L) was collected on 28/04/2014 from Station L4, pre-filtered through 100 μm mesh to remove large grazing zooplankton and aliquoted (2 L) into acid-washed sterilised glass 5 L conical flasks. To assess enrichments of specific taxa due to the addition of dDNA the treatments were a no addition control and an addition of dDNA (dDNA amended treatment), a further treatment of ^{13}C -labelled DNA was used to conduct RNA SIP experiments. The unlabelled dDNA amended treatments were also used as a ^{12}C control. All treatments were conducted in triplicate (*n* 3). Ambient D-DNA concentrations were quantified and diatom dDNA added to a final concentration of 1.29 $\mu\text{g L}^{-1}$ (3 x the ambient concentration at the time of sampling) in both the ^{12}C and ^{13}C dDNA amended treatments. The microcosms were incubated in the dark at 13°C (seawater temperature at Station L4 at the time of sampling) and aerated continually. At time point T0 before the addition of dDNA (0 hrs) and after 24 hrs, 660 mL seawater from each microcosm was filtered through a 0.2 μm cellulose nitrate membrane filter (Whatman, UK) and the filters stored at -80°C .

RNA was extracted from the filters in 1 mL Tri-reagent (Sigma Aldrich, UK). The extracted aqueous phase was added to 500 μL chloroform isoamyl alcohol (24:1) before vortexing and centrifuging at 10 000 *g* for 1 min. The resulting upper layer was recovered and the RNA precipitated with an equal volume of isopropanol and ammonium acetate (1:10) with 1 μL of 20 $\mu\text{g L}^{-1}$ molecular grade glycogen. The extracted RNA was cleaned using the RNeasy MinElute Cleanup Kit (Qiagen, UK) and treated twice with RQ1 RNase-Free DNase (Promega, UK) using the manufacturer's protocol. Absence of DNA was confirmed by a negative

PCR result using general bacterial primers as outlined below and the RNA stored at -80°C .

RNA stable isotope probing

RNA SIP was performed using cesium tri-fluoroacetate (CeTFA) gradients following established protocols (Whiteley et al. 2007; Taylor et al. 2013; Taylor and Cunliffe 2017). Gradients (5.1 mL) were prepared with 500 ng RNA, 1.75 mL formamide, 4.78 mL of a 2 g mL^{-1} CeTFA solution and molecular grade water to 5.1 mL. This produced a starting density of 1.8 g mL^{-1} . The tubes were centrifuged at 165 196 *g* (41 000 rpm) for 50 hrs in an ultracentrifuge (Beckman Coulter Optima L-100 XP ultracentrifuge rotor VTi 65.2) and gradients were fractionated into 420 μL fractions using displacement with sterile molecular grade water (Whiteley et al. 2007). Fraction density was determined from Refractive Index measured using digital refractometer (Bellingham Stanley). To the remaining gradient solution an equal volume of isopropanol and 1 μL of 20 $\mu\text{g L}^{-1}$ glycogen was added and precipitated for 1 h at -20°C . The tubes were centrifuged for 30 min at 12 000 *g* and then washed with 500 μL 75% ethanol. The RNA pellets were air dried in a laminar flow hood, re-suspended in RNase free water (20 μL) and the RNA quantified using the QuantiFluor® RNA system (Promega, Southampton, Hampshire, UK). Characterisation of all 'light' to 'heavy' SIP fractions was performed by quantification of buoyant densities and DGGE analysis of RT-PCR amplified 16S rRNA transcripts (see below). By comparing buoyant densities and DGGE fingerprints from unlabelled control incubations with labelled incubations, incorporation of ^{13}C -labelled dDNA into the bacterioplankton communities was determined (Whiteley et al. 2007; Taylor et al. 2013; Taylor and Cunliffe 2017). Specific 'heavy' gradient fractions from both the ^{12}C control and ^{13}C -labelled incubations were selected for further analysis using high-throughput sequencing as described below.

Controlling for amplification of bacterial DNA from dDNA additions

Using DNA as an added substrate means there is a potential that, with improper care, we could amplify organisms from our added material in downstream processes. The main step we took was to use RNA instead of DNA for diversity analysis and SIP. We took a series of other steps to ensure that our dDNA additions

were not amplified in downstream processes. The cultures of diatoms used were axenic (treated with antibiotics and filtered and washed through 3 µm filter) to minimise bacterial contamination of the dDNA additions. The DNA extraction from diatoms was twice RNase treated and the lack of RNA confirmed using the QuantiFluor® RNA System (Promega, Southampton, Hampshire, UK). From the experiment, RNA was extracted using tri-reagent, which minimises DNA contamination (Pinto *et al.* 2009). Extracted RNA was twice treated with DNase (30 min incubation) and the negative results confirmed by PCR of a bacteria 16S rRNA gene, as well as performing quantification of both DNA and RNA using respective QuantiFluor® high sensitivity kits. Furthermore, had any DNA been carried over into the ultracentrifugation, RNA and DNA have different buoyant densities and would therefore have been separated out. In sequencing of the no addition controls and T0 natural community we were able to determine that operational taxonomic units (OTUs) relating to the experiments were present in the original seawater sample and also in the control experiment that had no additions.

16S rRNA high-throughput sequencing and bioinformatics

RNA was reverse transcribed using the Omniscript Reverse Transcription kit according to the manufactures' instructions (Qiagen, UK) with the reverse primer PROK1492R (GGW TAC CTT GTT ACG ACT T) (Suzuki, Taylor and DeLong 2000). The V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F (5'-GTGCCAGCMGCGCGGTAA-3') and 806R (5'-CGACTACHVGGGTWTCTAAT-3') (Caporaso *et al.* 2011) in reactions using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) and ~10 ng cDNA. PCR conditions were as follows: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, and a final elongation step at 72°C for 5 min. Sequencing was performed on an Ion Torrent PGM (Life technologies, USA). Libraries were prepared using the Ion Xpress fragment library kit (Life technologies, USA) with template preparation using the Ion OneTouch2 400 bp v2 Template kit (Life technologies, USA). Sequencing was carried out using an Ion 400 bp sequencing kit on a 318v2 chip all accordance with the manufacturer's instructions. Signal processing and base calling was carried out using the TorrentSuite v4.4 software.

Sequences were analysed using a combination of USEARCH v7.0.1090 (32 Bit) (Edgar 2010) and QIIME v1.8.0 (Caporaso *et al.* 2010) as described previously (Taylor and Cunliffe 2017). Multiplexed files had barcodes removed and were quality filtered (low quality = expected error >0.5 and short sequences <200bp), length truncated (200bp) and converted to FASTA files. The FASTA files were de-replicated, abundance sorted and singleton sequences removed. OTUs were clustered using the UPARSE clustering algorithm (Edgar 2013). Chimeras were then filtered using UCHIME (Edgar *et al.* 2011) and the Gold database (Edgar 2010) as a reference. OTUs were mapped back to the original reads and an OTU table produced. Taxonomy was assigned to OTUs using the uclust method in QIIME v1.8.0 against a curated Greengenes reference database (release 13.8) (DeSantis *et al.* 2006). Before further analysis, singletons were removed as well as chloroplast plastid and archaeal sequences. OTU tables were then rarefied to 25 000 sequences per library. Weighted and un-weighted UniFrac distance matrices (Lozupone *et al.* 2011) were used to generate 2D principal component analysis plots. Sequence data are available from the European Nucleotide Archive (accession code PRJEB24573).

Statistical analysis

Permutational Multivariate Analysis of Variance (PERMANOVA) (999 permutations) was performed in QIIME using UniFrac distance matrices and OTU tables as inputs. All other statistical analysis was carried out using SPSS® (IBM®, USA).

RESULTS

Variation in seawater D-DNA at Station L4

The concentration of total D-DNA, which includes dissolved 'free' DNA, viruses and uncharacterised bound DNA, at Station L4 showed significant changes over the 16 week sampling period ($F_{16, 50} = 28.40$, $P < 0.001$), increasing from 0.09 µg L⁻¹ in early spring to a maximum of 1.5 µg L⁻¹ in the summer (Tukey, $P < 0.001$) (Fig. 1A). The mean concentration over the sampling period was 0.55 µg L⁻¹. A comparison between this study and other studies is shown in Table 1.

Patterns of nutrients and seawater temperature were typical for Station L4 (Smyth *et al.* 2010), with nutrients declining steeply during the spring diatom bloom. Throughout the sampling period, D-DNA concentration and surface seawater temperature showed a positive correlation (Spearman; $r = 0.705$; $P < 0.005$) (Fig. 1A; Table 1, Supporting Information) and a concurrent negative correlation with nutrients (Spearman; Nitrate $r = -0.64$, $P < 0.01$; Ammonia $r = -0.62$, $P < 0.05$; Silicate $r = -0.72$, $P < 0.005$; Phosphate $r = -0.67$, $P < 0.005$) (Fig. 1B).

Assessment of the changes in D-DNA concentrations and the abundance of specific phytoplankton groups showed strong positive correlations with pigmented dinoflagellates (phototrophs or mixotrophs) (Spearman, $r = 0.69$, $P < 0.005$), non-pigmented dinoflagellates (heterotrophs) (Spearman $r = 0.82$, $P < 0.000$) and diatoms (Spearman $r = 0.57$, $P < 0.02$). There was no significant correlation with chlorophyll-a over the study; however, there was a cross correlation between D-DNA and chlorophyll, with chlorophyll leading by 3 weeks followed by peaks in D-DNA (Spearman $r = 0.84$, $P < 0.001$) (Fig. 1C). A strong positive correlation was also seen between D-DNA concentration and high nucleic acid containing bacterioplankton (Spearman $r = 0.59$, $P < 0.02$) (Fig. 1D).

Impact of dDNA on bacterioplankton active diversity and dDNA assimilation

Surface seawater was collected for the microcosm experiments from Station L4 approximately 3 weeks after the spring diatom bloom when the water column was thermally stratified, with physicochemical parameters at the time of sampling typical for the time of year (Smyth *et al.* 2010). In the surface water above the thermocline, nutrients were depleted relative to values reported in the winter (Smyth *et al.* 2010) and the D-DNA concentration was 0.42 µg L⁻¹.

Weighted UniFrac distance matrices showed that the active bacterioplankton communities after 24 h were significantly different in the dDNA amended microcosms compared to the communities at T0 (i.e. un-amended seawater) and to the no addition control (i.e. no dDNA addition) after 24 h (PERMANOVA, $P < 0.05$) (Fig. 2A). No significant differences were seen between the treatments and time points based on the un-weighted (i.e. presence/absence) communities distance matrices (Fig. 2B).

At the time of sampling (T0) the active bacterioplankton communities were typical for the time of year at Station L4 (Taylor *et al.* 2014), dominated by the orders Flavobacteriales, Rhodobacterales, Oceanospirillales, Alteromonadales and the candidate

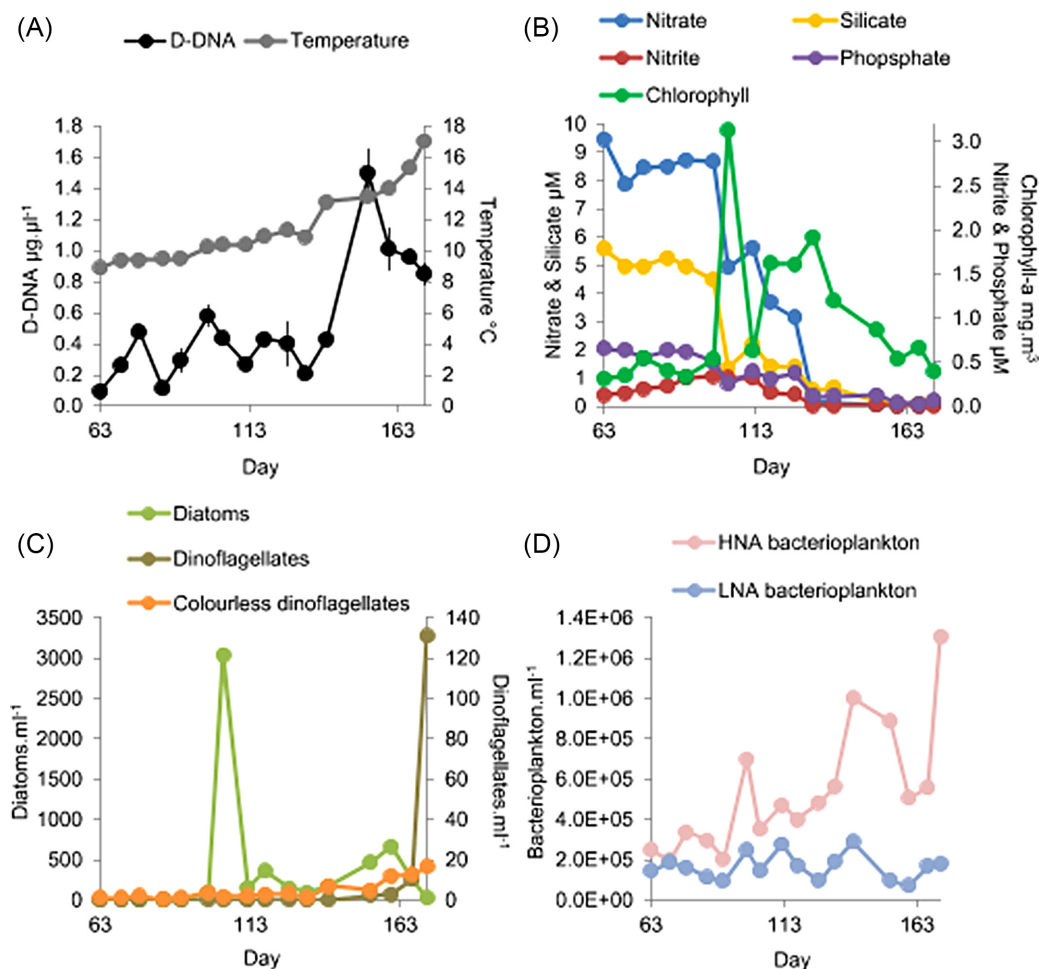


Figure 1. D-DNA (total DNA $<0.2 \mu\text{m}$) and other parameters measured at Station L4 during the study period (x axes are Julian days in 2014). (A) D-DNA concentration ($\mu\text{g } \mu\text{l}^{-1}$) \pm SE (n 3) and seawater temperature ($^{\circ}\text{C}$). (B) Nitrate, nitrite, silicate, phosphate (μM) and chlorophyll a ($\text{mg } \text{m}^{-3}$). (C) Diatoms, dinoflagellates, colourless dinoflagellates ($\text{cells } \text{mL}^{-1}$). (D) High-nucleic acid bacterioplankton and low-nucleic acid bacterioplankton ($\text{cells } \text{mL}^{-1}$).

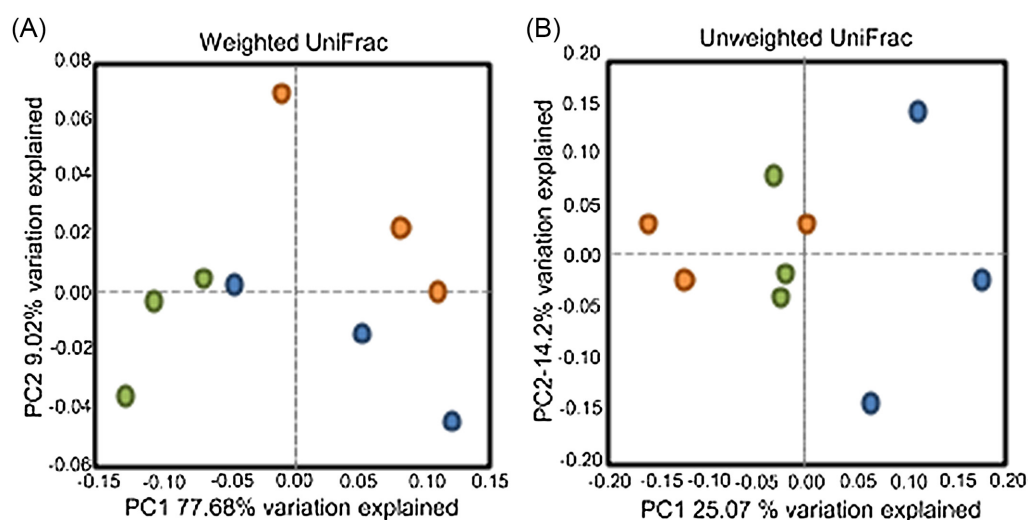


Figure 2. Principal coordinates analyses (PCoA) plot describing diversity of active bacterioplankton communities determined from 16S rRNA libraries using (A) weighted and (B) un-weighted UniFrac distance matrices generated from OTU (97% similarity) data. The blue circles represent the T0 samples, green circles represent the no addition control treatments after 24 h and the orange circles represent the dDNA amended treatments after 24 h.

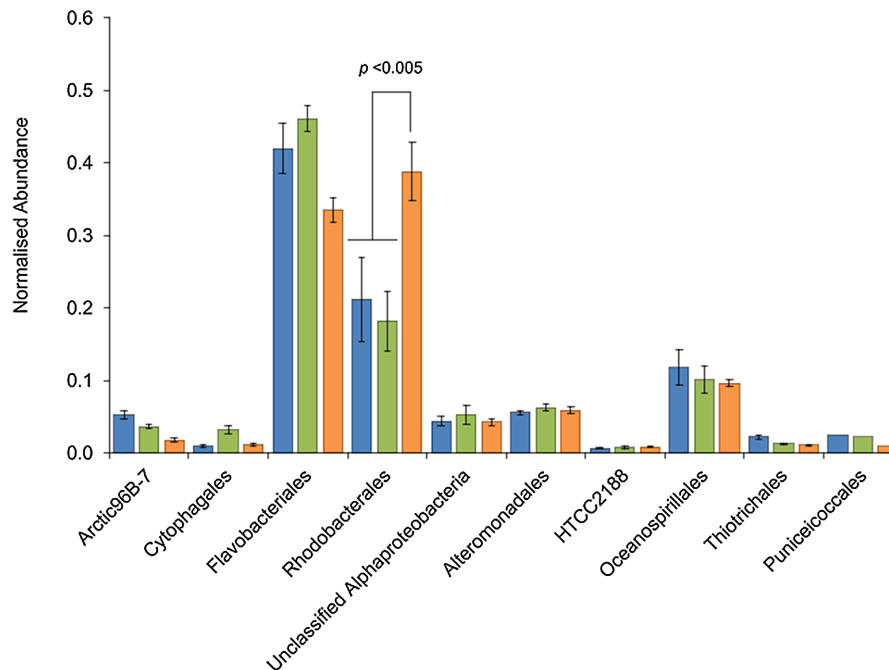


Figure 3. Normalised abundance of major bacterioplankton orders showing T0 blue bars, no addition control after 24 h green bars and dDNA amended treatments after 24 h orange. Values shown are means \pm standard error (n 3).

taxa Marine Group A-SAR406 (Arctic96B-7), representing 89% of the total sequences (Fig. 3). In the dDNA-amended treatments, the relative abundance of the order Rhodobacterales was significantly increased compared to the no addition controls ($P < 0.005$) (Fig. 3). Two OTUs made up the majority of the Rhodobacterales 16S rRNA reads (OTU_1, OTU_3) (~15%) (Fig. 4Bi). Both were affiliated to unclassified members of the Marine Roseobacter Clade (MRC). OTU_1 was most closely related to *Amylibacter* and OTU_3 was closer to *Halocynthiaibacter* based on partial 16S rRNA encoding genes (Fig. 4A). There was no significant enrichment of any other groups (Fig. 3).

At the OTU level there was significant enrichment in the dDNA amended treatments compared to the no addition control in four OTUs, OTU_3, OTU_37, OTU_410 and OTU_414 from the order Rhodobacterales. All four OTUs were from the MRC, with the highest difference in relative abundance in OTU_3 (ANOVA, Tukey $P < 0.004$) (Fig. 4Bi). Of the OTUs significantly enriched in the dDNA amended treatments, OTU_3 and OTU_37 also showed enrichment in the ^{13}C -dDNA RNA SIP heavy fractions compared to the ^{12}C control RNA SIP fractions (Fig. 4Bii) indicating that the taxa had assimilated the dDNA carbon.

DISCUSSION

The majority of studies on seawater DNA have focused on bacterial e.g. (Paul and Carlson 1984; DeFlaun, Paul and Jeffrey 1987) and viral sources e.g. (Jiang and Paul 1995; Brum 2005), with links to specific phytoplankton taxa generally overlooked. Although seawater D-DNA concentrations at Station L4 did not directly correlate with chlorophyll-a, D-DNA did show a cross correlation with chlorophyll-a, with concentrations of D-DNA increasing after the collapse of the spring diatom bloom. Dinoflagellates also showed a strong positive correlation with D-DNA, in particular during the period when D-DNA concentration was greatest at Station L4, suggesting that they may be an important direct or indirect source of DNA in seawater.

Dinoflagellates did not contribute greatly to total phytoplankton abundance in terms of cell numbers at Station L4 compared to other phytoplankton groups; however, dinoflagellates can contain large amounts of genomic DNA (up to 85 pg DNA.cell⁻¹) compared to other phytoplankton groups, such as diatoms (2 pg DNA.cell⁻¹) (Veldhuis, Cucci and Sieracki 1997). Many dinoflagellates can also be predatory, particularly non-pigmented cells (Sherr and Sherr 2007), and predation could also be a source of seawater DNA (Strom et al. 1997).

The constituent D-DNA pools at Station L4 (i.e. dissolved 'free' DNA, viruses and uncharacterised bound DNA) were not assessed individually. Other studies have shown that there can be substantial variation within the D-DNA pool. For example, at Station ALOHA dDNA can vary between 27% and 51% of the D-DNA pool and viruses between 49% and 63%, with uncharacterised bound DNA undetectable (Brum 2005). Viruses can act as both a cause of seawater dDNA release through viral lysis of phytoplankton hosts e.g. (Baudoux et al. 2006) and also form part of the total D-DNA pool when released (Brum 2005). Released viruses are protected by the capsid and are therefore not immediately available for degradation by DNases leading to longer turnover rates compared to dissolved 'free' DNA (Brum 2005). In this study, the D-DNA associated with specific phytoplankton taxa, such as the dinoflagellates, could be produced directly or as released viruses.

The positive correlation between HNA-containing bacterioplankton and D-DNA concentration at Station L4 suggests they could also contribute to the seawater DNA pool. However, as we show here, some bacterioplankton are able to assimilate dDNA and increase in abundance could also be caused in part by utilisation of dDNA as a growth substrate. The dominant bacterioplankton groups at Station L4 during the summer included Rhodobacterales, Flavobacteriales and Alteromonadales, which are categorised as HNA-containing bacterioplankton (Schattenhofer et al. 2011; Vila-Costa et al. 2012).

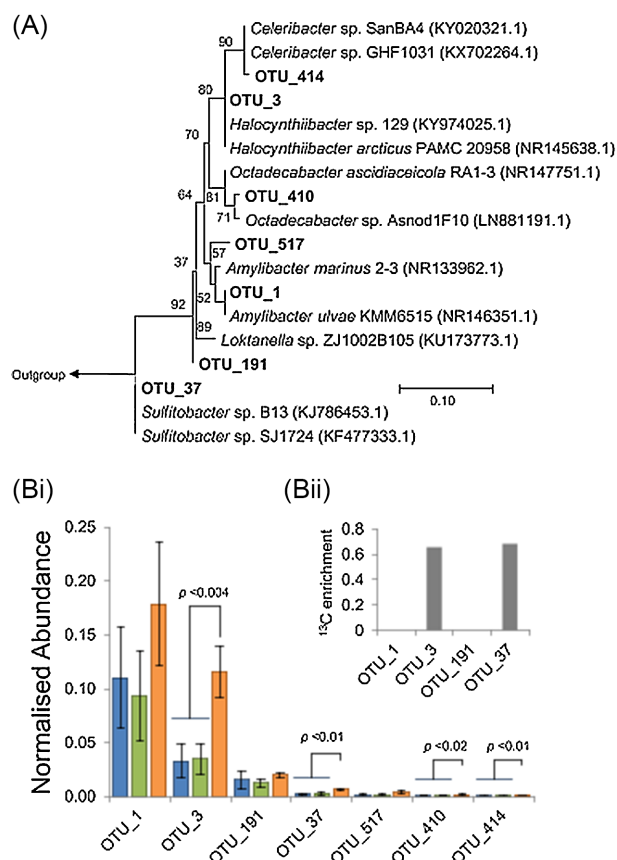


Figure 4. (A) Phylogenetic analysis of OTUs within the Rhodobacterales that showed increases in relative abundance in dDNA amended treatments compared to the no addition controls. The tree is based on a maximum likelihood method using a Tamura-Nei model. The numbers on the nodes indicate bootstrap percentages and the scale bar represents sequence divergence. (Bi) Normalised abundance of OTUs within the order Rhodobacterales showing enrichment, showing T0 blue bars, no addition control after 24 h green bars and dDNA amended treatments after 24 h orange. Values are means \pm standard error (n 3). (Bii) Comparison of the normalised relative abundance of 16S rRNA gene sequences from ^{13}C -labelled fraction and ^{12}C control fraction at the OTU level. Taxa >0.5 are those taxa enriched in the ^{13}C libraries relative to the ^{12}C control libraries, indicating that they had assimilated ^{13}C -labelled dDNA.

Rhodobacterales have been well established as ecologically linked to marine phytoplankton, and often increase in abundance during and following phytoplankton blooms in response to increased substrate availability (Gilbert et al. 2012; Teeling et al. 2012; Taylor et al. 2014). In this study, Rhodobacterales showed a significant increase in abundance due to dDNA additions with OTUs specifically from the MRC being enriched. Furthermore, two specific MRC OTUs were also shown to assimilate ^{13}C -dDNA in the RNA-SIP experiments, indicating that they utilise dDNA as a carbon source. These results corroborate previous culture-based studies that isolated dDNA-utilising Rhodobacterales (Lennon 2007). Laboratory-based experiments with the model MRC *Ruegeria pomeroyi* DSS-3 have shown that the bacterioplankton can utilise purines (a major component of nucleic acids) as a carbon and nitrogen source (Cunliffe 2015).

It could have been possible that those taxa with increased activity in dDNA-amended treatments but not enriched in the SIP experiment were utilising other elements in the dDNA such as nitrogen or phosphorus rather than carbon. Several studies have suggested that the MRC rely on organic and reduced nitrogen compounds (e.g. ammonium) as nitrogen sources (Moran

and Miller 2007; Newton et al. 2010; Chen 2012; Gifford et al. 2013) and are also able to utilise organic phosphorus sources (Moran and Miller 2007). Other studies have also linked members of the Rhodobacterales to degradation of DON in the form of dissolved protein using SIP (Orsi et al. 2016), suggesting they may be able to utilise a diverse range of organic compounds for growth. Whether various taxa within the MRC exhibit resource partitioning between them for different organic compounds remains to be determined.

In conclusion, in coastal marine waters MRC bacterioplankton in particular appear important in the degradation of phytoplankton-derived dDNA and assimilation of dDNA carbon. This observation further reinforces the 'master recycler' ecological role that the MRC hold proposed by Buchan et al. (2014). Bacterioplankton dDNA processing is ecologically and biogeochemically important because this is a potential mechanism through which phytoplankton-derived nitrogen and phosphorus-containing DOM compounds could be regenerated to sustain wider productivity throughout the summer once inorganic nutrients have been depleted by the spring bloom (Berman and Bronk 2003; Brum 2005; Berg and Jorgensen 2006; Karl and Björkman 2015). Future work should consider using molecular tools (metagenomics or metabarcoding) to sequence seawater dDNA to identify organisms contributing to the D-DNA pool, and also determine the underpinning biological mechanisms used by bacterioplankton to process seawater dDNA.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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